FORM PTO-1390
(REV. 11-94)

U S DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

ATTORNEY'S DOCKET NUMBER
7914-085-999

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/06185	July 3, 2000	July 6, 1999

10/019252

TITLE OF INVENTION
TAXANE DERIVATIVES AND PROCESSES FOR THE PREPARATION THEREOF

APPLICANT(S) FOR DO/EO/US
E. Bombardelli, B. Gabetta, A. Pontiroli

Applicant herewith submits to the United States Designated/ Elected Office (DO/EO/US) the following items under 35 U.S.C. 371:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the international Bureau).
   b. ☐ has been transmitted by the International Bureau
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2))

7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureaus.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 37(c)(3)).

9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)), unexecuted.

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information:
    ☒ Copy of European Search Report

INTERNATIONAL APPLICATION NO. PCT/EP00/06185	10/019252	INTERNATIONAL FILING DATE July 3, 2000	JC13 Rec'd PCT/PTO 28 DEC 2001

17. ☒ The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees as follows:

CLAIMS				
(1)FOR	(2)NUMBER FILED	(3)NUMBER EXTRA	(4)RATE	(5)CALCULATIONS
TOTAL CLAIMS	35 -20	15	X $18.00	$ 270.00
INDEPENDENT CLAIMS	17 -3	14	X $84.00	1,176.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $280.00	☐

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)): **CHECK ONE BOX ONLY**		
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) $710.00		
☐ No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $740.00		
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1,040.00		
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2) to (4) $100.00		
☒Filing with EPO or JPO search report $890.00		$ 890.00
Surcharge of $130.00 for furnishing the National fee or oath or declaration later than 20 30 mos. from the earliest claimed priority date (37 CFR 1.492(e)).		
10	TOTAL OF ABOVE CALCULATIONS	= 2,336.00
Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed also. (Note 37 CFR 1.9, 1.27, 1.28).		- $ 0.00
	SUBTOTAL	= 2,336.00
Processing fee of $130.00 for furnishing the English Translation later than 20 30 mos. from the earliest claimed priority date (37 CFR 1.492(f)).		+
0	TOTAL FEES ENCLOSED	$ 2,336.00

a. ☐ A check in the amount of $__ to cover the above fees is enclosed.

b. ☒ Please charge Deposit Account No. 16-1150 in the amount of $__ to cover the above fees. A copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 16-1150. A copy of this sheet is enclosed.

18. ☐ Other instructions

n/a

19. ☒ All correspondence for this application should be mailed to

PENNIE & EDMONDS LLP
1667 K Street, N.W.
Washington, D.C. 20006

20. ☒ All telephone inquiries should be made to

Paul E. Dietz (45,627)

Thomas G. Rowan	for Thomas G. Rowan	34,419	12-28-2001
NAME	SIGNATURE	REGISTRATION NUMBER	DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: E. BOMBARDELLI et al.

National Stage of PCT/EP00/06185

Filed: July 3, 2000

For: TAXANE DERIVATIVES AND PROCESSES FOR THE PREPARATION THEREOF

Group Art Unit: Unassigned

Examiner: Unassigned

Attorney Docket No.: 7914-085

PRELIMINARY AMENDMENT

Box PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please enter the following amendments and remarks into the file of the above-identified application prior to the examination thereof.

IN THE ABSTRACT

A marked up versions of the abstract showing insertions and deletions are included in Appendix A.

Please add the following abstract:

--A novel taxane derivative with anticancer activity, a process for its preparation and a process for the preparation of 14-β-hydroxy-1,14-carbonate-baccatine III and V derivatives 13-substituted by an isoserine residue.--

IN THE SPECIFICATION

Marked up versions of all revised paragraphs showing insertions and deletions are included in Appendix B.

Replace the paragraph starting at page 1, line 1 with the following text:

--TECHNICAL FIELD

The present invention relates to a novel taxane useful as chemotherapeutic agent, the pharmaceutical compositions containing it and a process for the preparation of 14-ß-hydroxy-l, 14-carbonate-baccatine III and V derivatives, substituted at the 13 position by an isoserine residue.--

Replace the paragraph starting at page 1, line 6 with the following text:

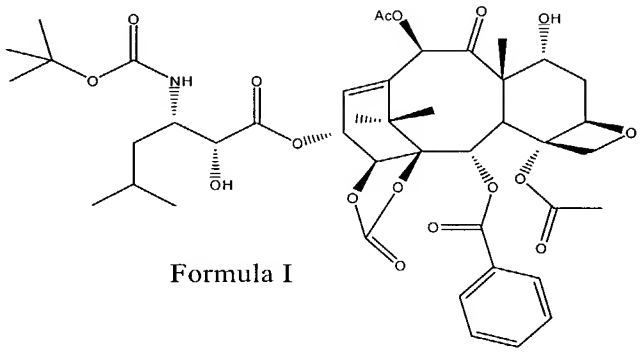
--BACKGROUND OF THE INVENTION

Taxanes are one of the most important classes of anticancer drugs recently developed. The remarkable effectiveness of Paclitaxel and of its analogue Docetaxel in the treatment of several tumors has focused research on substances with antimicrotubular activity. Taxanes are however characterized by a particular action mechanism, in that they promote the assembly of microtubules and inhibit tubuline depolymerization.--

Replace the paragraph starting at page 1, line 31 with the following text:

--SUMMARY OF THE INVENTION

It has now been found that the compound of formula (I), a 14ß-hydroxy-l, 14-carbonate-baccatine V derivative,



Formula I

has remarkable cytotoxic and anticancer activities, and is capable of overcoming the resistance of cell lines expressing the MDR phenotype.--

Replace the paragraph starting at page 2, line 14 with the following text:

--DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The compound of the invention differs from the prior art derivatives due to the hydroxyl at the 7- position, which in the present case is in alfa configuration. 13-(N-Boc-ß-Isobutylisoserinyl) -14ß--hydroxy-baccatine III 1, 14-carbonate, corresponding to the derivative referred to in US 5,705,508 as SB-T-101131, can be used as starting product for the preparation of compound (I) . In this case, said baccatine III derivative is either treated with DBU (diazabicyclo[5,4,0] 7-undecene) in methanol or THF or it is simply left in solution with methylene chloride or chlorinated solvents in the presence of aliphatic alcohols such as methanol, ethanol or propanol with basic allumine for a time ranging from one hour to 14 days. The compound having beta configuration at C-7, is converted at neutral or slightly basic pH to the more stable alfa isomer (baccatine V derivative).--

Replace the paragraph starting at page 28, line 1 with the following text:

--CLAIMS

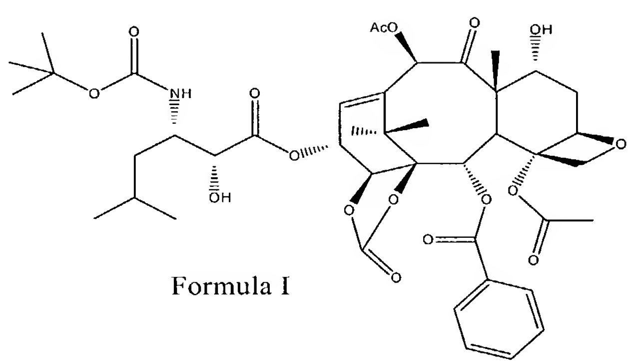
What is claimed is:--

IN THE CLAIMS

A complete listing of the currently pending claims is provided in Appendix C for the Examiners convenience.

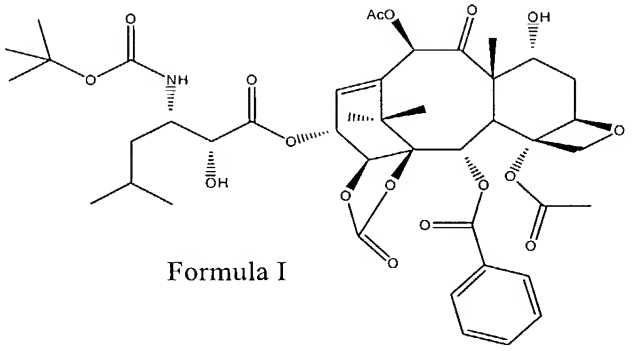
Please cancel claims 1-10 and add the following new claims:

11. (New) A compound of Formula I.



Formula I

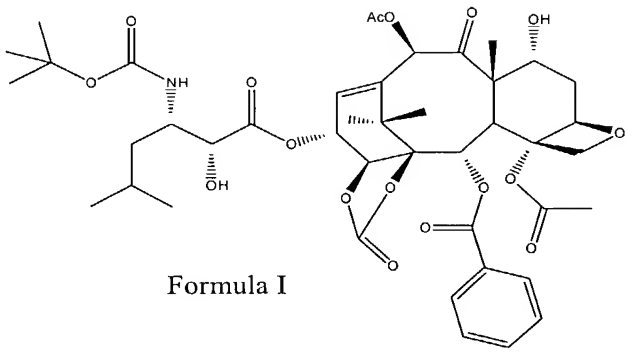
12. (New) A process for preparing a compound of Formula I,



Formula I

comprising reacting 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine III 1,14-carbonate with diazabicyclo[5,4,0] 7-undecene in methanol or THF.

13. (New) A process for preparing a compound of Formula I,



Formula I

comprising treating 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine III 1,14-carbonate with methylene chloride or chlorinated solvents in the presence of one or more aliphatic alcohols and basic allumine for from 1 hour to 14 days.

14. (New) The process of claim 13, wherein the one or more aliphatic alcohols are selected from methanol, ethanol, propanol, or a combination thereof.

15. (New) A process for preparing 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine III 1,14-carbonate or 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine V 1,14-carbonate, comprising:

a. reacting 14β-hydroxy-10-deacetylbaccatine III or 14β-hydroxy-10-deacetylbaccatine V with a silylating agent to provide a 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or a 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V;

b. reacting the 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or the 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V with phosgene to provide a 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or a 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V;

c. reacting the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V with a LiHMDS to provide a lithium salt of the 10-hydroxyl group of the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or a lithium salt of 10-hydroxyl group of the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V;

d. reacting the lithium salt of the 10-hydroxyl group of the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or the lithium salt of the 10-hydroxyl group of the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V with an acetylating agent to acetylate the 10-hydroxyl group to provide a 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine III or a 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine V;

e. reacting the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine III or the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine V with (4S,5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid to form a C-13 esterified 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine III or a C-13 esterified 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine V; and

f. removing the 7-triethylsilyl group from the C-13 esterified 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine V to provide a C-13 esterified 1,14 carbonate 7-hydroxyl 14β-hydroxy-10-acetylbaccatine III or a C-13 esterified 1,14 carbonate 7-hydroxy 14β-hydroxy-10-acetylbaccatine V; and

g. removing a dimethoxybenzylidene group from the C-13 esterified 1,14 carbonate 7-hydroxyl 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 1,14 carbonate 7-hydroxy 14β-hydroxy-10-acetylbaccatine V

to provide 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine III 1,14-carbonate or 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine V 1,14-carbonate.

16. (New) The process of claim 15, wherein the silylating agent is triethyl chlorosilane.

17. (New) The process of claim 15, wherein the 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or the 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V is reacted with phosgene by dissolving the 7-triethylsilylated derivative in a methylene chloride/pyridine mixture in a 3:1 ratio and then adding a toluene solution containing phosgene to the methylene chloride/pyridine mixture under a nitrogen atmosphere.

18. (New) The process of claim 15, wherein the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V is reacted with LiHMDS in anhydrous THF.

19. (New) The process of claim 15, wherein lithium salt of the 10-hydroxyl group of the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or the lithium salt of the 10-hydroxyl group of the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V is acetylating with acetyl chloride.

20. (New) The process of claim 15, wherein the the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine III or the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine V is reacted with the (4S,5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid in an anhydrous apolar organic solvent in the presence of a base and of a condensing agent.

21. (New) The process of claim 20, wherein the condensing agent is dicyclohexylcarbodiimide.

22. (New) The process of claim 15, wherein the 7-triethylsilyl group is removed from the 7-triethylsilyl group from the C-13 esterified 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine V with pyridinium fluoride in a acetonitrile/pyridine solution under nitrogen, and the dimethoxybenzylidene group is removed from the C-13 esterified 1,14 carbonate 7-hydroxyl 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 1,14 carbonate 7-hydroxy 14β-hydroxy-10-acetylbaccatine V in a methylene chloride solvent by addition of methanolic HCl followed by $NaHCO_3$.

23. (New) A process for preparing 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine III 1,14-carbonate or 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine V 1,14-carbonate, comprising:

a. acetylating the C-10 hydroxyl of 14β-hydroxy-10-deacetylbaccatine III or 14β-hydroxy-10-deacetylbaccatine V to provide 14β-hydroxy-10-acetylbaccatine III or 14β-hydroxy-10-acetylbaccatine V;

b. reacting the 14β-hydroxy-10-acetylbaccatine III or 14β-hydroxy-10-acetylbaccatine V with phosgene to provide a 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V;

c. silylating the C-7 hydroxyl of the 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or the 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V to provide a 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or a 7-silyl 1,14 carbonate derivative;

d. reacting the 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or the 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V with (4S,5R)-N-Boc-2- (2,4-dimethoxyphenyl) -4-isobutyl-l-oxazolidine-5- carboxylic acid to provide a C-13 esterified 7-silyl 1,14

carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or a C-13 esterified 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V;

e. removing the 7-triethylsilyl group from the C-13 esterified 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V to provide a C-13 esterified 7-hydroxy 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or a C-13 esterified 7-hydroxy 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V; and

f. removing a dimethoxybenzylidene group from the C-13 esterified 7-hydroxy 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 7-hydroxy 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V

to provide 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine III 1,14-carbonate or 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine V 1,14-carbonate.

24. (New) The process of claim 23, wherein the C-10 hydroxyl of 14β-hydroxy-10-deacetylbaccatine III or 14β-hydroxy-10-deacetylbaccatine V is acetylated with acetic anhydride in the presence of cerium, scandium, and/or ytterbium salts.

25. (New) The process of claim 24, wherein the salt is $CeCl_3 \cdot H_2O$.

26. (New) The process of claim 23, wherein 14β-hydroxy-10-acetylbaccatine III or 14β-hydroxy-10-acetylbaccatine V is reacted with phosgene by dissolving the 14β-hydroxy-10-acetylbaccatine III or 14β-hydroxy-10-acetylbaccatine V in a methylene chloride/pyridine mixture in a 3:1 ratio and then adding a toluene solution containing phosgene to the methylene chloride/pyridine mixture under a nitrogen atmosphere.

27. (New) The process of claim 23, wherein the C-10 hydroxyl of 14β-hydroxy-10-deacetylbaccatine III or 14β-hydroxy-10-deacetylbaccatine V is acetylated with acetyl chloride.

28. (New) The process of claim 23, wherein the 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or the 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V is reacted with (4S,5R)-N-Boc-2- (2,4-dimethoxyphenyl) -4-isobutyl-l-oxazolidine-5- carboxylic acid is reacted with (4S,5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid in an anhydrous apolar organic solvent in the presence of a base and a condensing agent.

29. (New) The process of claim 28, wherein the condensing agent is dicyclohexylcarbodiimide.

30. (New) The process of claim 23, wherein the triethylsilyl protective group is removed from the the C-13 esterified 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V with pyridinium fluoride in a acetonitrile/pyridine solution under nitrogen, and the dimethoxybenzylidene group is removed from the C-13 esterified 7-hydroxy 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 7-hydroxy 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V in a methylene chloride solvent by addition of methanolic HCl followed by $NaHCO_3$.

31. (New) A process for preparing (4S, 5R)-N-Boc-2-(2, 4-dimethoxyphenyl)-4- isobutyl-1-oxazolidine-5-carboxylic acid, comprising:

a. protecting an amino group of a leucinol with Boc to form N-Boc-L-leucinol;

b. converting of the N-Boc-L-leucinol into N-Boc-L-leucinal;

c. preparing a cyanhydrin nitrile from the N-Boc-L-leucinal;

d. transforming the cyanhydrine nitrile into a carboxylic acid;

e. forming of a methyl ester of the carboxylic acid from the carboxylic acid;

f. purifying the methyl ester of the carboxylic acid;

g. condensing the methyl ester of the carboxylic acid with 2,4-dimethoxybenzaldehyde dimethyl acetal to form (4S,

5R)-N-Boc-2-(2,4-dimethoxyphenyl) -4-isobutyl-l-oxazolidine-5-carboxylic acid methyl ester; and

h. transforming the (4S, 5R)-N-Boc-2-(2,4-dimethoxyphenyl) -4-isobutyl-l-oxazolidine-5-carboxylic acid methyl ester into the (4S, SR)-N-Boc-2-(2, 4-dimethoxyphenyl)-4- isobutyl-1-oxazolidine-5-carboxylic acid.

32. (New) A method of treating cancer in a patient in need thereof comprising administering to said patient a therapeutically effective amount of a compound of claim 1.

33. (New) The method of claim 32, wherein the compound is administered in an amount of from 50 to 500 mg/m^2.

34. (New) The compound 14β-hydroxy baccatine III.

35. (New) The compound 14β-hydroxy baccatine V.

36. (New) The compound 14β-hydroxy baccatine III 1,14 carbonate.

37. (New) The compound 14β-hydroxy baccatine V 1,14 carbonate.

38. (New) The compound 14-β-hydroxy-7-Tes-10-deacetylbaccatine III.

39. (New) The compound 14-β-hydroxy-7-Tes-10-deacetylbaccatine V.

40. (New) The compound 14-β-hydroxy-7-Tes-baccatine III.

41. (New) The compound 14-β-hydroxy-7-Tes-baccatine V.

42. (New) The compound 14-β-hydroxy-7-Tes-baccatine III 1,14-carbonate.

43. (New) The compound 14-β-hydroxy-7-Tes-baccatine V 1,14-carbonate.

44. (New) The compound (4S,5R)-N-Boc-2- (2,4-dimethoxyphenyl) -4-isobutyl-1-oxazolidine-5-carboxylic acid.

45. (New) A pharmaceutical composition comprising the compound of claim 1 and one or more pharmaceutically acceptable carriers and/or excipients.

REMARKS

New claims 11-45 are pending in this application for the Examiner's review and consideration. Applicants have amended the specification and claims to conform with U.S. patent practice and to more clearly recite the invention. As no new matter has been added herein, these changes should be entered.

Respectfully submitted, Paul E Dietz (45,627)

Date December 28, 2001 for Thomas G. Rowan

Thomas G. Rowan (Reg. No. 34,419)

PENNIE & EDMONDS LLP
1667 K Street, N.W.
Washington, DC 20006

(202) 496-4400

Appendix A

Changes to the Abstract

Please add the following abstract:

--A novel taxane derivative with anticancer activity, a process for its preparation and a process for the preparation of 14-β-hydroxy-1,14-carbonate-baccatine III and V derivatives 13-substituted by an isoserine residue.--

Appendix B

Changes to the Specification

The paragraph at page 1, line 1 is revised as follows:

--TECHNICAL FIELD

The present invention relates to a novel taxane useful as chemotherapeutic agent, the pharmaceutical compositions containing it and a process for the preparation of 14-ß-hydroxy-l, 14-carbonate-baccatine III and V derivatives, substituted at the 13 position by an isoserine residue.--

The paragraph at page 1, line 6 is revised as follows:

--BACKGROUND OF THE INVENTION

Taxanes are one of the most important classes of anticancer drugs recently developed. The remarkable effectiveness of Paclitaxel and of its analogue Docetaxel in the treatment of several tumors has focused research on substances with antimicrotubular activity. Taxanes are however characterized by a particular action mechanism, in that they promote the assembly of microtubules and inhibit tubuline depolymerization.--

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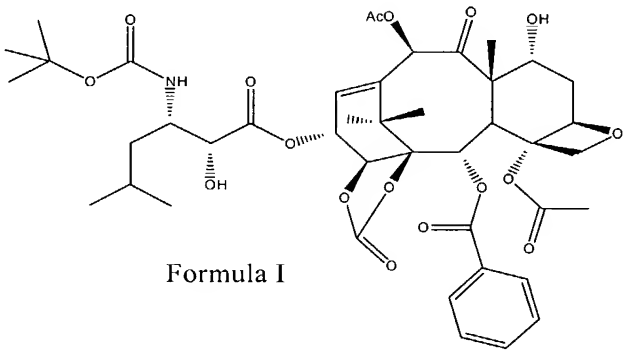
--SUMMARY OF THE INVENTION

It has now been found that the compound of formula (I), a 14ß-hydroxy-l, 14-carbonate-baccatine V derivative,



Formula I

has remarkable cytotoxic and anticancer activities, and is capable of overcoming the resistance of cell lines expressing the MDR phenotype.--

The paragraph at page 2, line 14 is revised as follows:

--DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The compound of the invention differs from the prior art derivatives due to the hydroxyl at the 7- position, which in the present case is in alfa configuration. 13-(N-Boc-ß-Isobutylisoserinyl) -14ß--hydroxy-baccatine III 1, 14-carbonate, corresponding to the derivative referred to in US 5,705,508 as SB-T-101131, can be used as starting product for the preparation of compound (I) . In this case, said baccatine III derivative is either treated with DBU (diazabicyclo[5,4,0] 7-undecene) in methanol or THF or it is simply left in solution with methylene chloride or chlorinated solvents in the presence of aliphatic alcohols such as methanol, ethanol or propanol with basic allumine for a time ranging from one hour to 14 days. The compound having beta configuration at C-7, is converted at neutral or slightly basic pH to the more stable alfa isomer (baccatine V derivative).--

The paragraph at page 28, line 1 is revised as follows:

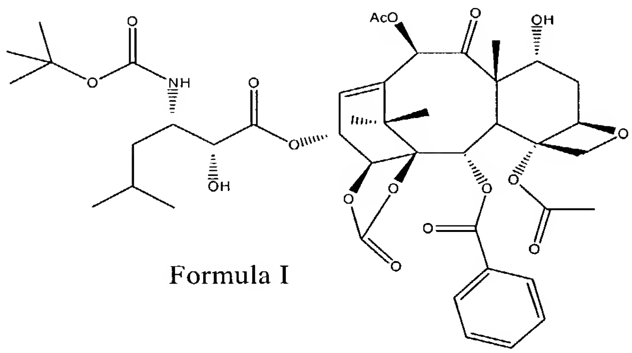
--CLAIMS

What is claimed is:--

Appendix C

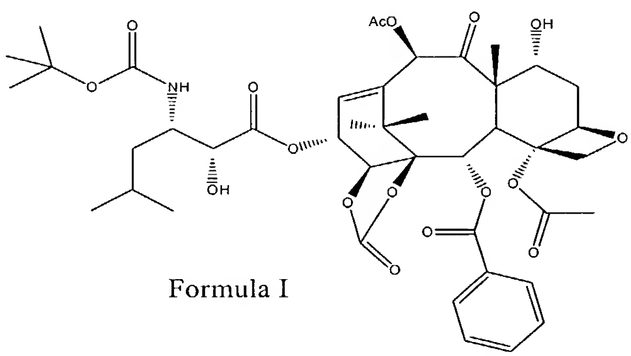
Currently Pending Claims

11. (New) A compound of Formula I.



Formula I

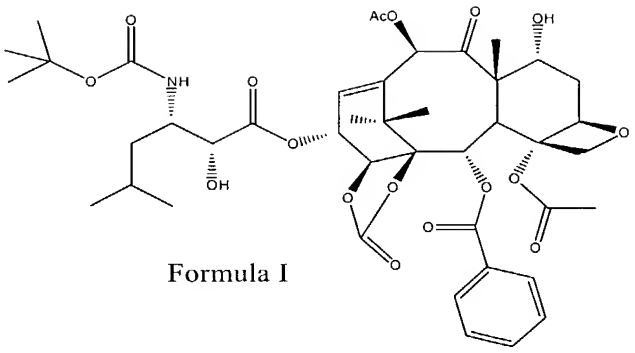
12. (New) A process for preparing a compound of Formula I,



Formula I

comprising reacting 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine III 1,14-carbonate with diazabicyclo[5,4,0] 7-undecene in methanol or THF.

13. (New) A process for preparing a compound of Formula I,



Formula I

comprising treating 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine III 1,14-carbonate with methylene chloride or chlorinated solvents in the presence of one or more aliphatic alcohols and basic allumine for from 1 hour to 14 days.

14. (New) The process of claim 13, wherein the one or more aliphatic alcohols are selected from methanol, ethanol, propanol, or a combination thereof.

15. (New) A process for preparing 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine III 1,14-carbonate or 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine V 1,14-carbonate, comprising:

a. reacting 14β-hydroxy-10-deacetylbaccatine III or 14β-hydroxy-10-deacetylbaccatine V with a silylating agent to provide a 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or a 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V;

b. reacting the 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or the 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V with phosgene to provide a 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or a 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V;

c. reacting the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V with a LiHMDS to provide a lithium salt of the 10-

hydroxyl group of the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or a lithium salt of 10-hydroxyl group of the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V;

d. reacting the lithium salt of the 10-hydroxyl group of the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or the lithium salt of the 10-hydroxyl group of the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V with an acetylating agent to acetylate the 10-hydroxyl group to provide a 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine III or a 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine V;

e. reacting the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine III or the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine V with (4S,5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid to form a C-13 esterified 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine III or a C-13 esterified 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine V; and

f. removing the 7-triethylsilyl group from the C-13 esterified 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine V to provide a C-13 esterified 1,14 carbonate 7-hydroxyl 14β-hydroxy-10-acetylbaccatine III or a C-13 esterified 1,14 carbonate 7-hydroxy 14β-hydroxy-10-acetylbaccatine V; and

g. removing a dimethoxybenzylidene group from the C-13 esterified 1,14 carbonate 7-hydroxyl 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 1,14 carbonate 7-hydroxy 14β-hydroxy-10-acetylbaccatine V

to provide 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine III 1,14-carbonate or 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine V 1,14-carbonate.

16. (New) The process of claim 15, wherein the silylating agent is triethyl chlorosilane.

17. (New) The process of claim 15, wherein the 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or the 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine

V is reacted with phosgene by dissolving the 7-triethylsilylated derivative in a methylene chloride/pyridine mixture in a 3:1 ratio and then adding a toluene solution containing phosgene to the methylene chloride/pyridine mixture under a nitrogen atmosphere.

18. (New) The process of claim 15, wherein the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V is reacted with LiHMDS in anhydrous THF.

19. (New) The process of claim 15, wherein lithium salt of the 10-hydroxyl group of the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine III or the lithium salt of the 10-hydroxyl group of the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-deacetylbaccatine V is acetylating with acetyl chloride.

20. (New) The process of claim 15, wherein the the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine III or the 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine V is reacted with the (4S,5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid in an anhydrous apolar organic solvent in the presence of a base and of a condensing agent.

21. (New) The process of claim 20, wherein the condensing agent is dicyclohexylcarbodiimide.

22. (New) The process of claim 15, wherein the 7-triethylsilyl group is removed from the 7-triethylsilyl group from the C-13 esterified 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 1,14 carbonate 7-triethylsilyl 14β-hydroxy-10-acetylbaccatine V with pyridinium fluoride in a acetonitrile/pyridine solution under nitrogen, and the dimethoxybenzylidene group is removed from the C-13 esterified 1,14 carbonate 7-hydroxyl 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 1,14 carbonate 7-hydroxy 14β-hydroxy-10-acetylbaccatine V in a methylene chloride solvent by addition of methanolic HCl followed by $NaHCO_3$.

23. (New) A process for preparing 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine III 1,14-carbonate or 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine V 1,14-carbonate, comprising:

a. acetylating the C-10 hydroxyl of 14β-hydroxy-10-deacetylbaccatine III or 14β-hydroxy-10-deacetylbaccatine V to provide 14β-hydroxy-10-acetylbaccatine III or 14β-hydroxy-10-acetylbaccatine V;

b. reacting the 14β-hydroxy-10-acetylbaccatine III or 14β-hydroxy-10-acetylbaccatine V with phosgene to provide a 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V;

c. silylating the C-7 hydroxyl of the 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or the 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V to provide a 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or a 7-silyl 1,14 carbonate derivative;

d. reacting the 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or the 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V with (4S,5R)-N-Boc-2- (2,4-dimethoxyphenyl) -4-isobutyl-l-oxazolidine-5- carboxylic acid to provide a C-13 esterified 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or a C-13 esterified 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V;

e. removing the 7-triethylsilyl group from the C-13 esterified 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V to provide a C-13 esterified 7-hydroxy 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or a C-13 esterified 7-hydroxy 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V; and

f. removing a dimethoxybenzylidene group from the C-13 esterified 7-hydroxy 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 7-hydroxy 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V

to provide 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine III 1,14-carbonate or 13-(N-Boc-β-isobutylisoserinyl)-14β-hydroxy-baccatine V 1,14-carbonate.

24. (New) The process of claim 23, wherein the C-10 hydroxyl of 14β-hydroxy-10-deacetylbaccatine III or 14β-hydroxy-10-deacetylbaccatine V is acetylated with acetic anhydride in the presence of cerium, scandium, and/or ytterbium salts.

25. (New) The process of claim 24, wherein the salt is $CeCl_3 \cdot H_2O$.

26. (New) The process of claim 23, wherein 14β-hydroxy-10-acetylbaccatine III or 14β-hydroxy-10-acetylbaccatine V is reacted with phosgene by dissolving the 14β-hydroxy-10-acetylbaccatine III or 14β-hydroxy-10-acetylbaccatine V in a methylene chloride/pyridine mixture in a 3:1 ratio and then adding a toluene solution containing phosgene to the methylene chloride/pyridine mixture under a nitrogen atmosphere.

27. (New) The process of claim 23, wherein the C-10 hydroxyl of 14β-hydroxy-10-deacetylbaccatine III or 14β-hydroxy-10-deacetylbaccatine V is acetylated with acetyl chloride.

28. (New) The process of claim 23, wherein the 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or the 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V is reacted with (4S,5R)-N-Boc-2- (2,4-dimethoxyphenyl) -4-isobutyl-l-oxazolidine-5- carboxylic acid is reacted with (4S,5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid in an anhydrous apolar organic solvent in the presence of a base and a condensing agent.

29. (New) The process of claim 28, wherein the condensing agent is dicyclohexylcarbodiimide.

30. (New) The process of claim 23, wherein the triethylsilyl protective group is removed from the the C-13 esterified 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 7-silyl 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V with pyridinium fluoride in a acetonitrile/pyridine solution

under nitrogen, and the dimethoxybenzylidene group is removed from the C-13 esterified 7-hydroxy 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine III or the C-13 esterified 7-hydroxy 1,14 carbonate derivative of 14β-hydroxy-10-acetylbaccatine V in a methylene chloride solvent by addition of methanolic HCl followed by $NaHCO_3$.

31. (New) A process for preparing (4S, 5R)-N-Boc-2-(2, 4-dimethoxyphenyl)-4- isobutyl-1-oxazolidine-5-carboxylic acid, comprising:

a. protecting an amino group of a leucinol with Boc to form N-Boc-L-leucinol;

b. converting of the N-Boc-L-leucinol into N-Boc-L-leucinal;

c. preparing a cyanhydrin nitrile from the N-Boc-L-leucinal;

d. transforming the cyanhydrine nitrile into a carboxylic acid;

e. forming of a methyl ester of the carboxylic acid from the carboxylic acid;

f. purifying the methyl ester of the carboxylic acid;

g. condensing the methyl ester of the carboxylic acid with 2,4-dimethoxybenzaldehyde dimethyl acetal to form (4S, 5R)-N-Boc-2-(2,4-dimethoxyphenyl) -4-isobutyl-l-oxazolidine-5-carboxylic acid methyl ester; and

h. transforming the (4S, 5R)-N-Boc-2-(2,4-dimethoxyphenyl) -4-isobutyl-l-oxazolidine-5-carboxylic acid methyl ester into the (4S, SR)-N-Boc-2-(2, 4-dimethoxyphenyl)-4- isobutyl-1-oxazolidine-5-carboxylic acid.

32. (New) A method of treating cancer in a patient in need thereof comprising administering to said patient a therapeutically effective amount of a compound of claim 1.

33. (New) The method of claim 32, wherein the compound is administered in an amount of from 50 to 500 mg/m^2.

34. (New) The compound 14β-hydroxy baccatine III.

35. (New) The compound 14β-hydroxy baccatine V.

36. (New) The compound 14β-hydroxy baccatine III 1,14 carbonate.

37. (New) The compound 14β-hydroxy baccatine V 1,14 carbonate.

38. (New) The compound 14-β-hydroxy-7-Tes-10-deacetylbaccatine III.

39. (New) The compound 14-β-hydroxy-7-Tes-10-deacetylbaccatine V.

40. (New) The compound 14-β-hydroxy-7-Tes-baccatine III.

41. (New) The compound 14-β-hydroxy-7-Tes-baccatine V.

42. (New) The compound 14-β-hydroxy-7-Tes-baccatine III 1,14-carbonate.

43. (New) The compound 14-β-hydroxy-7-Tes-baccatine V 1,14-carbonate.

44. (New) The compound (4S,5R)-N-Boc-2- (2,4-dimethoxyphenyl) -4-isobutyl-1-oxazolidine-5-carboxylic acid.

45. (New) A pharmaceutical composition comprising the compound of claim 1 and one or more pharmaceutically acceptable carriers and/or excipients.

TAXANE DERIVATIVES AND PROCESSES FOR THE PREPARATION THEREOF

The present invention relates to a novel taxane useful as chemotherapeutic agent, the pharmaceutical compositions containing it and a process for the preparation of 14-ß-hydroxy-1,14-carbonate-baccatine III and V derivatives, substituted at the 13 position by an isoserine residue.

Taxanes are one of the most important classes of anticancer drugs recently developed. The remarkable effectiveness of Paclitaxel and of its analogue Docetaxel in the treatment of several tumors has focused research on substances with antimicrotubular activity. Taxanes are however characterized by a particular action mechanism, in that they promote the assembly of microtubules and inhibit tubuline depolymerization.

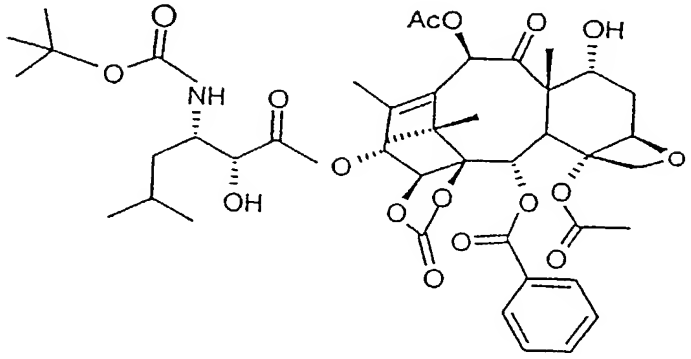
The main drawbacks of the taxanes presently used are: (a) insolubility in water, making mandatory the use of specific carriers which can cause hypersensitization reactions, (b) toxicities which limit dosages, (c) development of resistance mechanisms. Cell resistance to taxanes has been related to the MDR phenotype ("multidrug resistance") mediated by the P-glycoprotein transporter, by tubuline alterations, and by changes in the expression of apoptotic regulatory proteins.

In order to find novel active molecules having higher solubility and better tolerability, 14ß-hydroxy-10-deacetylbaccatine III and V taxane derivatives have been synthesized.

Some derivatives of 14-hydroxy baccatine III substituted at the 13- position by isoserine residues are disclosed in US 5,705,508, together with a process for the preparation thereof.

It has now been found that the compound of formula

(I), a 14ß-hydroxy-1,14-carbonate-baccatine V derivative,



(I)

has remarkable cytotoxic and anticancer activities, and is capable of overcoming the resistance of cell lines expressing the MDR phenotype.

Said compound differs from the derivatives described in the above mentioned American Patent due to the hydroxyl at the 7- position, which in the present case is in alfa configuration. 13-(N-Boc-ß-Isobutylisoserinyl)-14ß-hydroxy-baccatine III 1,14-carbonate, corresponding to the derivative referred to in US 5,705,508 as SB-T-101131, can be used as starting product for the preparation of compound (I). In this case, said baccatine III derivative is either treated with DBU (diazabicyclo[5,4,0] 7-undecene) in methanol or THF or it is simply left in solution with methylene chloride or chlorinated solvents in the presence of aliphatic alcohols such as methanol, ethanol or propanol with basic allumine for a time ranging from one hour to 14 days. The compound having beta configuration at C-7, is converted at neutral or slightly basic pH to the more stable alfa isomer (baccatine V derivative).

Alternatively, compound (I) can be prepared with a process which also allows to prepare the corresponding beta epimer at C-7.

Said process (A) comprises the following steps:

a) transformation of 14ß-hydroxy-10-

deacetylbaccatine III or V into the derivative triethylsilylated at the 7- position;

b) preparation of the 1,14 carbonate derivative from the product of step (a);

c) selective acetylation of the 10- hydroxyl;

d) reaction of the product of step (c) with (4S, 5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid;

e) cleavage of the triethylsilyl and dimethoxybenzylidene protective groups from the product of step (d).

According to a preferred embodiment of process (A), triethylchlorosilane is used as silylating agent in step (a), whereas the 1,14 carbonate derivative in step (b) is prepared using phosgene in toluene in a 3:1 methylene chloride/pyridine solution under nitrogen atmosphere. In the following step (c) 14-ß-hydroxy-10-deacetylbaccatine III or V 7-Tes-1,14-carbonate is salified with LiHMDS in anhydrous THF, thereby obtaining the 10-hydroxy derivative lithium salt, which is subsequently acetylated with acetyl chloride. The condensation reaction between 14-ß-hydroxy-7-Tes-1,14-carbonate-baccatine III or V and (4S, 5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid (step (d)) is carried out in anhydrous apolar organic solvent, in the presence of a base and a condensing agent such as dicyclohexylcarbodiimide (DCC).

Finally, in step (e) triethylsilyl is removed with pyridinium fluoride in acetonitrile/pyridine solution under nitrogen, whereas the dimethoxybenzylidene group is removed in methylene chloride solvent by addition of methanol HCl and subsequently of $NaHCO_3$.

The step sequence of the process described can be inverted thus obtaining the final product in as much comparable yields. Said alternative process (B) comprises

the following steps:

a') selective acetylation of the hydroxyl at C-10 of 14ß-hydroxy-10-deacetylbaccatine III or V;

b') preparation of the 1,14 carbonate derivative from the product of step (a')

c') silylation of the hydroxyl at C-7;

d') reaction of the product of step (c') with (4S, 5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid;

e') cleavage of the triethylsilyl and dimethoxybenzylidene protective groups from the product of step (d').

The latter process involves a number of advantages such as the possibility to obtain the desired synton (1,14-carbonate-7-Tes-baccatine III or V) without chromatographic purifications, merely by crystallization.

According to a preferred embodiment, the selective acetylation of step (a') is carried out with acetic anhydride in the presence of cerium, scandium, ytterbium salts, preferably $CeCl_3.7H_2O$, whereas the remaining steps are carried out as indicated above.

The present invention also comprises, as intermediate products of the process for the preparation of 14ß-hydroxy-1,14-carbonate baccatine III or V, the following compounds: 14ß-hydroxy baccatine III or V, 14ß-hydroxy baccatine III or V 1,14 carbonate, 14-ß-hydroxy-7-Tes-10-deacetylbaccatine III or V, 14-ß-hydroxy-7-Tes-baccatine III or V, 14-ß-hydroxy-7-Tes-baccatine III or V 1,14-carbonate.

A further aspect of the invention relates to a process for the preparation of (4S, 5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid, according to the following scheme:

SCHEME

BocNH
OH
O
O
OH
275.34
$C_{13}H_{25}NO_5$
IX
BocHN
OH
CO_2Me
IX A (2R, 3S)
IX B (2S, 3S)
BocN
423.50
$C_{22}H_{33}NO_7$
X
409.47
$C_{21}H_{31}NO_7$
XI
BocHN
OH
COOH
VIII
H_2N
COOH
OH
•HCl
VII
BocHN
CN
OH
VI
H_2N
OH
II
BocHN
CHO
III
IV
BocHN
HSO_3
ONa
V

Said process comprises the following steps:

a) protection of the amino group of leucinol with Boc;

b) transformation of N-Boc-L-leucinol into N-Boc-L-leucinal;

c) preparation of the cyanhydrin of the product from step (b);

d) transformation of the cyanhydrin nitrile into the corresponding carboxylic acid;

e) formation of the carboxylic acid methyl ester;

f) purification of the (2R, 3S)-3-(N-Boc)amino-2-hydroxy-5-methylhexanoic acid methyl ester;

g) condensation of the product of step (f) with 2,4-dimethoxybenzaldehyde dimethyl acetal;

h) transformation of the (4S, 5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid methyl ester into the corresponding carboxylic acid.

According to a preferred embodiment, in step (a) leucinol is reacted with Boc-anhydride, and subsequently oxidized to aldehyde in DMSO/CH_2Cl_2 solvent using oxalyl chloride at a temperature below -60°C, neutralizing the formed acid with triethylamine, or oxidizing it with sodium hypochlorite at -2 to -5°C. The cyanhydrin of step (c) is prepared by substituting the sulfonic group of the intermediate 1-hydroxy-2-(N-Boc)amino-4-methylpentanesulfonate by the cyanide ion. The cyanhydrin is then hydrolyzed to the corresponding carboxylic acid in step (d) by refluxing in concentrated hydrochloric acid.

In step (e), (2R/S,3S)-3-(N-Boc)amino-2-hydroxy-5-methylhexanoic acid is converted in the corresponding methyl ester by reaction with diazomethane in ethereal solution. In step (f), diastereomer (2R, 3S) is purified by crystallization from cyclohexane or an hexane/toluene

mixture. Step (g) is carried out in THF in the presence of pyridinium p-toluenesulfonate removing the developed methanol; after completion of the reaction, pyridinium p-toluenesulfonate is neutralized with bicarbonate. In step (h), the ester is hydrolysed in a methanol/water mixture with potassium carbonate. The reaction mixture is subsequently acidified and the final product is extracted with methylene chloride.

The invention also comprises (4S, 5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid as an intermediate for the synthesis of baccatine III and V derivatives substituted at the 13- position by a N-Boc-ß-isobutylserinyl residue.

The novel taxane of the present invention showed a strong anticancer activity against cancerous cells of breast, lung, ovary, colon, prostate, kidney, pancreas, and also against cells resistant to the known anticancer drugs such as adriamycin, vinblastine and platinum derivatives.

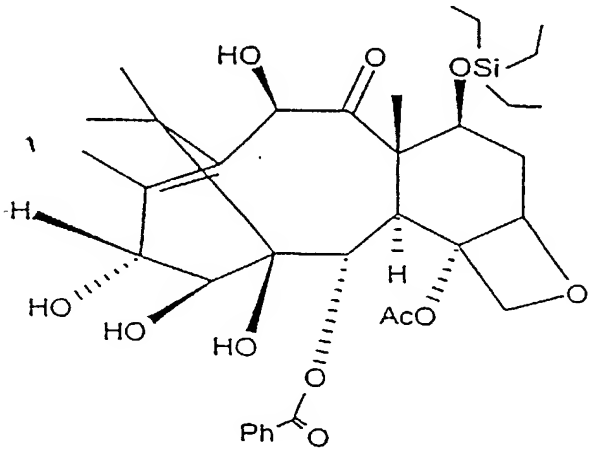
Therefore, the invention relates to pharmaceutical formulations containing an effective amount of the compound of the invention, together with pharmacologically acceptable carriers and excipients. More particularly, the compound can be formulated in the form of tablets, powders, granulates, capsules, injectables, solutions, suppositories, emulsions, dispersions, and the like. For the intravenous administration, mixtures of Chremophor L and ethanol, polysorbate and ethanol or liposome formulations prepared with natural or synthetic phosphatidylcholine, or mixtures of natural phospholipids in the presence of cholesterol are mainly used; for the oral administration, soft-gelatin capsules in which the product is solubilised in polysorbates, PEG or mixtures thereof, optionally in the presence of phospholipids, are preferably prepared. Compound (I) can be administered to

humans at concentrations from 50 to 500 mg/m^2.

The following examples illustrate the invention in greater detail.

Example 1: Synthesis of 13-(N-Boc-ß-isobutylserinyl)-14ß-hydroxybaccatine III, 1,14 carbonate

43.26 g of 14ß-hydroxy-deacetylbaccatine III together with 22.3 ml of N-methyl-imidazole were dissolved in 230 ml of DMF in a 500 ml glass round-bottom flask; this solution was added under strong stirring at room temperature in 1h with 14 ml of triethylchlorosilane. When the reaction was over, the reaction mixture was poured into 2L of water under strong stirring. An abundant precipitate formed, which was left at 4°C overnight. The precipitate was then filtered, thoroughly washing with water and subsequently with n-hexane. After drying under vacuum 48.1 g of 7-Tes-10-deacetylbaccatine III (XII) were obtained containing a small percentage of the 7,10-derivative, having the following chemical-physical characteristics:



(XII)

^{1}H NMR ($CDCl_3$ 200 MHz): δ (ppm) = 0.55 (6H, t, J = 7.8 Hz, 7-OTES CH_2), 0.94 (9H, q, J= 7.8 Hz, 7-OTES CH_3), 1.18

(3H, s, C16H_3), 1.20 (3H, s, C17H_3), 1.77 (3H, s, C19H_3), 1.90 (1H, ddd, J = 2.4, 10.8, 13.2 Hz, C6Hß), 2.12 (3H, d, J = 1.6 Hz, C18H_3), 2.31 (3H, s, 4-OCOCH_3), 2.48 (3H, ddd, J = 14.3, 9.8, 6.5 Hz, C6Hα), 2.73 (1H, d, J = 5.5 Hz, OH) 3.79 (1H, d, J = 7.1 Hz, C3H), 4.20 (1H, dd, J = 1.0, 8.3 Hz, C20Hß), 4.31 (1H, d, J = 8.6 Hz, C20Hα), 4.39 (1H, dd, J = 6.4, 10.7 Hz, C7H), 4.77 (1H, d, J = 5.8 Hz, C14H), 4.94 (1H, dd, J = 2.1, 9.7 Hz, (C5H), 5.05 (1H, m, C13H), 5.13 (1H, d, J = 1.9 Hz, C10H), 6.05 (1H, d, J = 7.3 Hz, C2H), 7.41-8.09 (5H, m, Ph).

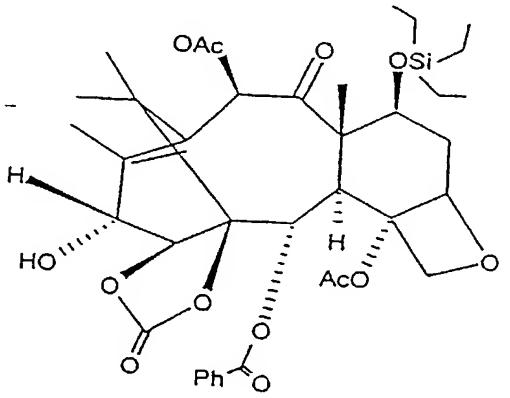
Mass Spectrum (NH_3, DEP/CI, positive ions): *(m/z)* 718 [$(M+NH_4)^+$, 100%], 701 [$M+H)^+$, 39%].

The resulting compound was dissolved in 300 ml of a methylene chloride/pyridine 3:1 mixture under nitrogen atmosphere; this solution was added under with stirring to a phosgene solution (214 ml of a 1.9M solution in toluene) precooled at -10°C, keeping temperature from -5 to -10°C during the addition.

The reaction mixture was stirred for 30', then shaken with 700 ml of a $NaHCO_3$ saturated solution keeping temperature below or at 2°C. The phases were separated and the organic phase was washed to remove pyridine. The organic phase was dehydrated over $MgSO_4$ and concentrated to dryness. 46.6 g of 10-deacetylbaccatine III 7-Tes-1,14-carbonate were obtained which could be directly used for the following reactions.

31 g of the compound were dissolved in 250 ml of strictly anhydrous THF; the solution was cooled at -50°C and added with 48 ml of a 1M LiHMDS solution in 2 minutes and stirred for 20 minutes at the same temperature. 3.7 g of acetyl chloride were added during 40 min, with stirring. The reaction temperature was left to raise to 0°C keeping stirring for 2h. Upon completion of the reaction, the mixture was treated with a NH_4Cl saturated solution and

diluted with ethyl acetate. The phases were separated and the aqueous solution was diluted with ethyl acetate until exhaustion of the product. The combined organic phases were washed with water then dried over $MgSO_4$ and concentrated to dryness. 33 g of 14ß-hydroxy-7-Tes-1,14-carbonate-baccatine III were obtained, impure due to the compounds of the preceding reactions. This compound was chromatographed on silica gel eluting the pure product with an ethyl acetate/CH_2Cl_2 9:1 mixture. 30 g of the desired product (XIII) were obtained, having the following characteristics:



(XIII)

^1H NMR ($CDCl_3$ 200 MHz): δ (ppm) = 0.55 (6H, t, *J* = 7.8 Hz, 7-OTES CH_2), 0.95 (9H, q, *J* = 7.8 Hz, 7-OTES CH_3), 1.16 (3H, s, C16H_3), 1.32 (3H, s, C17H_3), 1.77 (3H, s, C19H_3), 1.88 (1H, ddd, *J* = 2.4, 10.8, 13.2 Hz, C6Hß), 2.21 (3H, d, *J* = 1.6 Hz, C18H_3), 2.19 (3H, s, 10-OCOCH_3), 2.31 (3H, s, 4-OCOCH_3), 2.48 (3H, ddd, *J* = 14.3, 9.8, 6.5 Hz, C6Hα), 2.73 (1H, d, *J* = 5.5 Hz, OH) 3.72 (1H, d, *J* = 7.1 Hz, C3H), 4.20 (1H, d, *J* = 8.3 Hz, C20Hß), 4.31 (1H, d, *J* = 8.6 Hz, C20Hα), 4.46 (1H, dd, *J* = 6.4, 10.7 Hz, C7H), 4.79 (1H, d, *J* = 5.8 Hz, C14H), 4.94 (1H, dd, *J* = 2.1, 9.7 Hz, (C5H), 5.02 (1H, m, C10H), 5.05 (1H, m, C13H), 6.09 (1H, d, *J* = 7.3 Hz, C2H), 7.41-8.09 (5H, m, Ph).

Mass Spectrum (NH_3, DEP/CI, positive ions): *(m/z)* 759 [$(M+NH_4)^+$, 19%], 743 [$M+H)^+$, 100%].

20 g of 14ß-hydroxy-7-Tes-1,14-carbonate-baccatine III together with a 300 ml of strictly anhydrous toluene were

placed in a 1L round-bottom flask, 10 g of (4S, 5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazoli-dine-5-carboxylic acid and 2 g of N,N-dimethylaminopyridine (DMAP) and 9.5 g of dicyclohexylcarbodiimide (DCC) dissolved in CH_2Cl_2 were added. The reaction mixture was refluxed for 3h, then cooled, the ureic product was precipitated off and mother liquors were washed with a $NaHCO_3$ saturated solution to remove the unreacted acid, then with diluted hydrochloric acid to remove DMAP and finally again with $NaHCO_3$ to neutrality. The organic phase was concentrated to dryness to obtain 41.5 g of product which could be directly used in the subsequent step.

40 g of this compound were deprotected in two steps, by removing first Tes and then 2,4-dimethoxybenzaldehyde. 40 g of the compound were dissolved in 100 ml of an acetonitrile/pyridine mixture (80:100) under nitrogen and cooled at 0°C; 13 ml of pyridinium fluoride were added and the whole was left under stirring for 24 h. The solution was poured into 2L of water and the product was filtered and dried under vacuum.

The residue was dissolved in 60 ml of methylene chloride and this solution was added with 40 ml of 0.6N HCl in methanol under strong stirring and at 0°C. The reaction mixture was left for 2h under stirring, then diluted with 150 ml of methylene chloride and shaken with a $NaHCO_3$ solution adjusting pH to 6-7. The organic phase was concentrated to dryness and the residue was crystallized from acetone hexane. After drying, 16 g of 13-(N-Boc-ß-isobutylisoserinyl)-14ß-hydroxybaccatine-1,14-carbonate were obtained, having the following chemico-physical and spectroscopical characteristics:

Formula: $C_{44}H_{57}NO_{17}$.

Aspect: white powder.

Melting point: 245°C

Table 1: Chemical shifts (ppm) ^{1}H NMR in $CDCl_3$ solution (200 MHz)

H	Ppm, multiplicity (Hz)	H	Ppm, multiplicity (Hz)
2	6.09-d (7.8)	2'	4.30-dd (6.4; 3.2)
3	3.68-d (7.4)	3'	4.08-m
5	4.91-dd (9.7; 2.5)	4'a	1.21-m
6α	2.52-ddd (14.8; 9.8; 6.9)	4'b	1.43-m
6ß	1.86-m	5'	1.65-m
7	4.37-m	6'	0.96-d (6.3)
10	6.25-s	7'	0.95-d (6.3)
13	6.44-d (broad, 6.9)	4-OCOC$\underline{H}_3$	2.40-s
14	4.84-d (6.9)	10-OCOC$\underline{H}_3$	2.22-s
16	1.25-s	Boc	1.35-s
17	1.32-s	o-benzoyl	8.01-m
18	1.87-d (1.6)	m-benzoyl	7.46-m
19	1.69-s	p-benzoyl	7.58-m
20α	4.27-d (8.4)	3-NH	4.72-d (9.0)
20ß	4.20-d (8.4)		

Mass Spectra: (NH_3, **DEP/CI, positive ions):** *(m/z)* 889 [$(MNH_4)^+$], 832 [$(MNH_4-(CH_3)_3C)^+$], 772 [$(MNH_4-BocNH_2)^+$].

(NH_3, **DEP/CI, negative ions):** *(m/z)* 871 (M^-), 260 (side chain)

Infrared Spectrum (KBr disc): 3521, 3321, 2971, 2953, 1826, 1762, 1706, 1526, 1366, 1238, 1165, 1072, 723 cm^{-1}

UV Spectrum (MeOH): 231, 276 and 284 nm;

-$E_{1\%}$ at 231 nm = 180.99

-$E_{1\%}$ at 276 nm = 14.094

-$E_{1\%}$ at 284 nm = 12.182

Example 2: Synthesis of 13-(N-Boc-ß-isobutylserinyl)-14ß-hydroxybaccatine V, 1,14 carbonate

5 g of 13-(N-Boc-ß-isobutylserinyl)-14ß-hydroxybaccatine III, 1,14 carbonate were dissolved in 500 ml of toluene under argon atmosphere, completely deoxygenating the solution; 80 mg of DBU (diazabicyclo[5,4,0]7-undecene) were added and the reaction mixture was refluxed for 1 hour under argon atmosphere. The solution was diluted with 100 ml of ethyl acetate and washed with water. The organic phase was evaporated to dryness to obtain 4.5 g of 13-(N-Boc-ß-isobutylserinyl)-14ß-hydroxybaccatine V 1,14 carbonate having the following chemical-physical and spectroscopical characteristics:

Formula: $C_{44}H_{57}NO_{17}$

Aspect: white powder

Melting point: 245°C

Table 2: Chemical shifts (ppm) ^{13}C NMR in $CDCl_3$ solution (50.308 MHz)

C	ppm, multiplicity	C	ppm, multiplicity
9	201.8-s	8	58.2-s
1'	172.6-s	3'	51.2-d
4-O<u>C</u>OCH$_3$	170.5-s	3	44.6-d
10-O<u>C</u>OCH$_3$	170.2-s	15	41.3-s
2-<u>C</u>OPh	164.3-s	4	39.9-t
<u>C</u>=O (Boc)	155.8-s	6	34.9-t
<u>C</u>=O (carbonate)	151.4-s	(<u>C</u>H$_3$)$_3$C Boc	27.7-q
12	139.4-s	17	25.5-q
11	133.1-s	16	22.6-q
(Me)$_3$<u>C</u>(Boc)	80.0-s	4-OCO<u>C</u>H$_3$	22.0-q
5	83.8-d	10-OCO<u>C</u>H$_3$	20.2-q
1	87.7-s	5'	24.3-d
4	80.0-s	6'	22.7-q
2	69.0-d	7'	21.6-q
20	75.5-t	18	14.6-q
2'	73.3-d	19	9.8-q
7	71.2-d	q-benzoyl	127.5-s
10	74.3-d	o-benzoyl	129.5-d
13	74.1-d	m-benzoyl	128.6-d
14	79.1-d	p-benzoyl	133.7-d

Table 3: Chemical shift (ppm) ^1H NMR in $CDCl_3$ solution (200 MHz)

H	Ppm, multiplicity (Hz)	H	Ppm, multiplicity (Hz)
2	6.18 d (7.9)	2'*	4.75 d (8.6)
3	3.80 d (7.8)	3'	4.01 m
5	4.93 dd (7.8. 4.8)	4'a	1.25 m
6	2.23 m	4'b	1.48 m
7	3.76 m	5'	1.67 m
10	6.79 s	6'	0.99 d (6.4)
13	6.44 d (6.7)	7'	0.97 d (6.4)
14	4.88 d (7.0)	4-OCOC$\underline{H}_3$	2.58 s
16	1.29 s	10-OCOC$\underline{H}_3$	2.20 s
17	1.31 s	Boc	1.37 s
18	1.87 d (1.5)	o-benzoyl	8.06 m
19	1.71 s	m-benzoyl	7.49 m
20	4.38 s	p-benzoyl	7.61 m
		3'-NH*	4.60 d (11.2)

* Can be reversed

Table 4: Chemical shift (ppm) ^{13}C NMR in $CDCl_3$ solution (50.308 MHz)

C	Ppm, multiplicity	C	Ppm, multiplicity
9	206.1 s	8	58.2 s
1'	173.1 s	3'	52.0 d
4-O<u>C</u>OCH_3	172.7 s	3	40.4 d
10-O<u>C</u>OCH_3	169.3 s	15	41.5 s
2-<u>C</u>OPh	165.1 s	4'	40.6 t
<u>C</u>=O(Boc)	156.6 s	6	35.2 t
<u>C</u>=O(Carbonate)	152.1 s	($\underline{C}H_3$)$_3$C (Boc)	28.4 q
12	137.6 s	17	25.4 q
11	134.0 s	16	22.4 q
(Me)$_3$<u>C</u>(Boc) §	81.7 s	4-OCO<u>C</u>H_3	22.7 q
5	82.7 d	10-OCO<u>C</u>H_3	18.6 q
1	88.5 s	5'	25.1 d
4 §	80.7 s	6'	23.4 q
2	69.9 d	7'	20.9 q
20	77.2 t	18	15.2 q

(Continued)

Table 4: (Continued)

Chemical shift (ppm) ^{13}C NMR in $CDCl_3$ solution (50.308 MHz)

2'°	74.6	d	19^	16.2	q
7°	77.6	d	q-benzoyl	128.3	s
10°	74.2	d	o-benzoyl	130.2	d
13°	76.0	d	m-benzoyl	128.2	d
14	79.9	d	p-benzoyl	134.4	d

*, §, °, ^ = Can be reversed

Mass Spectrum (TSP+): (*m/z*) 872 (MH^+); 816 (MH^+-$(CH_3)_2C=CH_2$); 772 (816-CO_2); 756 (816-AcOH); 712 (772-AcOH)

Infrared Spectrum (KBr disc): 3450, 2963, 1813, 1740, 1702, 1247, 1091, 710 cm^{-1}

UV Spectrum (MeOH): 200 e 230 nm

-$E_{1\%}$ at 200 nm = 370.9

-$E_{1\%}$ at 230 nm = 193.2

Example 3: Preparation of (4S, 5R)-N-Boc-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid

Preparation of N-Boc-L-leucinol (III):

46.8 g of L-leucinol II (400 mmol) were dissolved in 300 ml of CH_2Cl_2 in a 2l three-necked round-bottom flask equipped with mechanical stirrer, thermometer and dropping funnel. The stirred solution was then added drop by drop at room temperature with the solution of Boc anhydride (87.2 g, 400 mmol) in CH_2Cl_2 (100 mL) in 90 minutes. During the addition of the first 25% of Boc-anhydride, the reaction was exothermic and it reached 20-30°C yielding a slurry which turned clear after stirring at room temperature for a further three hours. The whole was left at room temperature overnight. The solvent was evaporated under high vacuum to obtain the desired product as a thick oil in a quantitative yield (87 g). The product was subsequently treated without further purifications.

Preparation of N-Boc-L-leucinal (IV)

A solution of oxalyl chloride (26.274 mL, 300 mmol) in 130 ml of methylene chloride precooled at -60/-65°C was slowly added with DMSO (28.4 mL, 400 mmol).

The solution turned clear when the addition of DMSO was completed. After 20 minute stirring at the same temperature the reaction mixture was subsequently treated with a solution of alcohol III (43.7 g, 200 mmol) in CH_2Cl_2 (200 mL) for 25 min. keeping temperature below -60°C. During the addition of the alcohol the reaction mixture

became cloudy, and a white precipitate formed. After 20-25 minutes of stirring at the same temperature a solution of triethylamine (112 mL, 800 mmol) in CH_2Cl_2 (100 mL) was added dropwise in 40 minutes keeping temperature between -68 and -62°C. The reaction mixture was then stirred at between -60 and -65°C for a further 50 minutes. TLC of the reaction mixture carried out using 8% methanol in CH_2Cl_2 as eluent detected no starting product.

The cold solution was then poured into 800 ml of an iced solution containing 68 g (0.5 mol) of $KHSO_4$. The organic layer was separated and the aqueous phase extracted with CH_2Cl_2 (100 mL). The combined organic phases were washed with aqueous $KHSO_4$ (5%, 1x200 mL), brine (100 mL, 50 mL) and concentrated to half volume (~250 mL). Said material was used directly in the subsequent step.

Aldehyde (V) bisulfite compound derivative

The methylene chloride solution of the aldehyde (IV) in a 2l three-necked round-bottom flask equipped with mechanical stirrer, thermometer and dropping funnel was treated in 10 minutes and at -5°C with a sodium solution bisulfite (41.7 g, 400 mmol) in water (200 mL) and subsequently with n-Bu_4NHSO_4 (678 mg, 2 mmol). The solution was cooled to -5°C. The reaction mixture was stirred at -5 to -0°C for 5-6 hours and subsequently overnight at room temperature. The aqueous phase containing compound V was separated and washed with CH_2Cl_2 (2 x 20 mL).

(2-Cyano-3-(N-Boc)-amino-5-methyl-hexanol (VI)

The above aqueous solution (~250 mL) was added with CH_2Cl_2 (120 mL) and the reaction mixture was cooled to 0-5°C on an ice bath. Solid KCN (15 g, 230 mmol) was subsequently added to the reaction mixture and the solution was stirred at room temperature overnight. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with

brine (1x50 mL), dried over $MgSO_4$ and evaporated to obtain the product as a colourless viscous liquid (43 g). The product had $[\alpha]_D$ 51.11 (c=2, MeOH) and was an about 2:1 mixture of the VI 2(R),3(S) and 2(S),3(S) derivatives. The yield was 89% compared with the starting L-leucinol.

(2RS,3S)-3-Amino-2-hydroxy-5-methylhexanoic acid (VII)

The mixture of the above crude nitrile VI (43 g) was treated with 150 ml of concentrated HCl (37%) (150 mL) and refluxed overnight to give the crude acid VII*. The hydrochloric acid excess was removed by rotatory evaporator and the residue was evaporated with water (100 mL) to remove HCl. The residue was then dissolved in 150 ml of water and added with 100 ml of acetone, then treated with 33 ml of a 6.25M NaOH solution to adjust pH to 5. A further amount of acetone (500 mL) was then added to the solution which was left to stand overnight at 4°C. The precipitated solid was subsequently filtered and the solid cake was washed with acetone and dried under vacuum to give crude acid VII (6.5 g) containing an about 3:1 mixture of 2(R),3(S) and 2(S),3(S) derivatives of compound VI.

The filtrate was evaporated and water was added to adjust the volume of the solution to 75 mL.

Acetone (1 L) was then added to the solution which was left to stand overnight at 4°C in refrigerator. The precipitated solid was then filtered and the solid cake was washed with acetone and dried under vacuum to give a second amount of product (18 g) containing solid NaCl with an about 1:1 mixture of 2(R),3(S) and 2(S),3(S) derivatives of VII.

The first product VII recovered (22.5 g) was heated in water (120 mL) without obtaining a complete dissolution and then cooled in ice and filtered to obtain 12.5 g of acid VII still contaminated by about 10% of undesired 2(R),3(S) derivative of VII. This product was dried and mixed with

the above 1:1 mixture of the second crop crystals (total ~27 g).

(2RS,3S)-3-(N-Boc)Amino-2-hydroxy-5-methylhexanoic acid (VIII)

(A) The crude acid VI 2(R),3(S), about 90% purity, (2.5 g, 77.6 mmol) was dissolved in a water - THF 1:1 mixture (80 mL), then triethylamine (13.5 mL) and subsequently Boc anhydride (18.5 g, 85 mmol) were added to the reaction mixture, the whole solution was stirred for 40 hours at room temperature. The solvent was evaporated by rotatory evaporator, 60 ml of water and 60 ml of ethyl acetate were added keeping the whole under stirring. The aqueous phase was separated and extracted with ethyl acetate (30 mL). The combined organic phases were extracted with 10% aqueous sodium carbonate (30 mL, 20 mL). The basic extract was then combined with an aqueous phase acidified with 2M hydrochloric acid (~55 mL) to adjust pH of the solution to 2. Acid VIII was then extracted from the aqueous phase with ethyl acetate (3x40 mL) and the heteroacetic extracts were washed with water (20 mL), dried ($MgSO_4$) and evaporated to give the crude VIII Boc derivative as syrup (20 g, 99%).

(B) The crude acid VII 2R,3S, with purity of about 50%, contaminated by NaCl (27 g), was dissolved in a water - dioxane 1:1 mixture (120 mL). Triethylamine (20 mL) was then added to the reaction mixture, then Boc anhydride (26.16 g, 120 mmol). The solution was stirred for 40 hours at room temperature. The solvent was evaporated by rotatory evaporator and water (100 mL) and ethyl acetate (100 mL) were added to the residue keeping stirring for a further few minutes. The organic phase was separated and extracted with 10% aqueous sodium carbonate (45 mL, 30 mL). The sodium carbonate extracts were then combined with the aqueous phase, acidified with 1M hydrochloric acid (~165

mL) and extracted with ethyl acetate (3x60 mL), afterwards washed with water (30 mL), dried ($MgSO_4$) and evaporated to give the crude VII Boc as syrup (16 g), consisting of a 1:1 mixture of the 2R,3S and 2S,3S isomers.

(2R,3S)-3-(N-Boc)Amino-2-hydroxy-5-methylhexanoic acid methyl ester (IX)

Diazomethane was prepared from diazald following the process reported in T.H. Black [Aldrichimica Acta, 16, 3 (1983)].

(A) A solution of the crude acid VIII (20 g, 56.6 mmol) in CH_2Cl_2 (75 mL) was slowly added to a cold diazomethane ethereal solution (-77 mmol) and the mixture was left for two hours on ice bath. The colour of the solution in that step turned white thus indicating that most diazomethane had been adsorbed. The solution was then concentrated and the residue crystallized from a mixture of toluene (20 mL) and hexane (70 mL). After cooling overnight in refrigerator at 4°C, the crystals of the pure IXA 2R,3S derivative were collected by filtration. The yield was 15 g. The mother liquors gave about 5 g of a 1:1 isomeric mixture.

(B) Using the same procedure, a 1:1 mixture of acid VIII (16 g) was transformed into a 1:1 mixture of IXA and IXB esters. The material from mother liquors (5 g from step A) was added and the material was combined and separated by column chromatography using hexane-ethyl acetate as eluent (9:1 to 7:3). Ninhydrine was used as developer for the TLC plates. The apolar compound, Rf 0.75 (hexanoethyl acetate : 7:3) was identified as the desired ester IXA (2R,3S), which was recrystallized from cyclohexane to give IXA as colorless needles (8 g) m.p. 95-96°C, $[\alpha]_D$ 72,4° (c=1, MeOH).

The polar compound, Rf 0.5 (hexane-ethyl acetate 7:3) was identified as IXB (2S,3S), and was recrystallized from

cyclohexane to give 10 g of IXB as colorless needles.

2,4-dimethoxybenzaldehydedimethyl acetal

A mixture of 2,4-dimethoxybenzaldehyde (41.25 g, 0.25 mols), anhydrous trimethyl orthoformate (50 mL) and ammonium nitrate (2 g dissolved in 20 ml of methanol) was refluxed for 6 hours (^{1}HNMR of the reaction mixture showed a 65-70% conversion). At first, the hot reaction mixture was a clear solution, but as the reaction progressed the solid precipitated. A second portion of anhydrous trimethyl orthoformate (20 mL) was added and part of methanol was distilled off.

When the temperature of the reaction mixture reached 95-100°C, all the solid dissolved in the flask. The solution was cooled to room temperature and added with anhydrous Na_2CO_3 (5 g), stirring for 30 min. Subsequently the solution was filtered and the residue was distilled by fractional distillation under vacuum at 0.25 mmHg. The first fraction at low temperature mainly consisted of the trimethyl orthoformate excess and the second fraction, which distilled as colourless oil at 175-180°C, was the desired acetal. Yield: 37 g (70%).

(4S,5R)-N-Boc-2-(2,4-Dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid methyl ester (X)

A solution of (2R, 3S)-3-(N-Boc)amino-2-hydroxy-5-methylhexanoic acid methyl ester (IXA) (34.375 g, 125 mmol) in anhydrous THF (150 ml) was added with distilled 2,4-dimethoxybenzaldehyde dimethyl acetal (30 g, 142 mmol) and subsequently pyridinium p-toluenesulfonate (Py.Tos; 400 mg).

The solution was heated under mild reflux in a 500 ml three-necked flask equipped with a Dean-Stark separator. After about 6 hours under reflux, about 60 ml of THF containing methanol generated during the reaction were removed. A sample was taken for ^{1}H NMR analysis (in $CDCl_3$).

The peak at δ = 1.41 ppm disappeared (1) and a novel peak appeared at δ = 1.24 ppm for the protected methyl ester (2). After 6 hour reflux, the conversion was about 70-75%.

A fresh aliquot of anhydrous THF (50 ml) was added, then an amount of 2,4-dimethoxybenzaldehyde acetal (5,0 g; 24 mmol). The reaction mixture was refluxed for a further 2.5 hours, during which time about 50 ml of THF were removed using the Dean-Stark apparatus. The subsequent ^1H NMR analysis showed the complete transformation of the starting material.

The reaction mixture was added with a $NaHCO_3$ saturated aqueous solution (15 ml) and the mixture was stirred for 15 minutes to neutralize Py.Tos. t-Butyl methyl ether (85 ml) and water (15 ml) were subsequently added and the organic phase was separated. The aqueous phase was extracted with t-butyl methyl ether (20 ml) and the combined organic phases were washed with water (30 ml) and evaporated to a residue (66 g) of crude product X.

Hydrolysis of ester X to give acid XI

The crude ester X (22 g, 42 mmol) was dissolved in 100 ml of methanol and added with water (50 ml) containing 8.7 g of potassium carbonate. After stirring overnight at room temperature, the reaction was considered completed by TLC monitoring (toluene-ethyl acetate: 4.5:1). TLC analysis was confirmed by ^1H NMR analysis, checking the disappearance of the methyl ester peak.

Methanol was evaporated at a temperature not above 40°C under vacuum (about 60 g residue) and water (150 ml) was added to the residue. The aqueous suspension was extracted with ethyl acetate (5x50 ml) to remove the benzaldehyde and benzaldehyde dimethyl acetal excess. 90 ml of methylene chloride were added to the aqueous phase, the mixture was cooled on ice bath and the diphasic system was treated with about 125 ml of 1M $NaHSO_4$ (pH = 3) under

strong stirring. The phases were separated and the aqueous phase was extracted with methylene chloride (75 ml). The combined methylene chloride extracts were washed with water (30 ml), brine (30 ml) and dried over $MgSO_4$. The solution was then kept at -60°C until next use. The yield in the final product as colourless solid was of 16 g, about 93% based on the starting product.

Example 4: Preparation of 14ß-hydroxy-7-Tes baccatine III 1,4 carbonate

A solution of 11.2 g of 10-deacetyl-14-hydroxybaccatine III in 50 ml of dry tetrahydrofuran was added with 0.72 g of $CeCl_3.7H_2O$ and 7.3 ml of acetic anhydride. The reaction mixture was stirred at room temperature for 5 hours; during this time the mixture became homogeneous. 10 g of ice were added and the whole was stirred for 1 hour. Tetrahydrofuran was evaporated off under vacuum and the residue was diluted with 200 ml of H_2O. The precipitate was filtered and dried under vacuum in the presence of P_2O_5: the product was crystallized from ethyl acetate to obtain 10 g of 14-hydroxybaccatine III having the following characteristics:

Mp: 236-8°C; IR (KBr): 3474, 1739, 1400. 1240. 1090. 1049 cm^{-1}.

^1H NMR ($CDCl_3$, 200 MHz); 8.07 (d, J = 8 Hz, Bz), 7.55 (d, J = 8 Hz; Bz), 7.44 (t, J = 8 Hz, Bz), 6.31 (s, H-10), 5.80 (d, J = 7 Hz, H-2), 4.97 (br d, J = 8 Hz, H-5), 4.73 (br, d, J = 4 Hz, H-13), 4.41 (m, H-7),, 4.24 (d, J = 4 Hz, H-14), 4.20 (d, J = 7 Hz, H-20a), 4.06 (d, J = 7 Hz, H-20b), 3.89 (J 0 (Hz, H-3), 2.29 (s, OAc), 2.22 (s, OAc), 2.04 (s, H-18), 1.66 (s, H-19), 1.25, 1.11 (s, H-16 and H-17).

In a four-necked flask equipped with stirrer, dropping funnel, thermometer and reflux condenser cooled to -12°C, were placed 52.8 ml of a 1.9M solution of phosgene in

toluene. This solution was dropwise added with 11.6 g of 14-hydroxy baccatine III dissolved in 53 ml of methylene chloride and 17.5 ml of pyridine under stirring in 30 minutes. Temperature was kept between -6 and -10°C. After 30 minutes 50 ml of $NaHCO_3$ saturated solution were added under stirring keeping a tight control of the temperature. After warming to room temperature, the phases were separated. The aqueous phase was contraextracted with methylene chloride and the organic phases were washed with 45 ml of 2N HCl adjusting pH to about 1. The organic phase was washed with 0.1N HCl and then with $NaHCO_3$, then dried over Na_2SO_4 and evaporated to dryness to quantitatively obtain 11.5 g of 14-hydroxybaccatine-1,14 carbonate.

11.5 g of 14-hydroxybaccatine-1,14 carbonate were dissolved in 50 ml of DMF and 1.1 equivalents of chlorotriethylsilane and 3 equivalents of N-methyl-imidazole were added at room temperature. After completion of the reaction, the mixture was poured into 500 ml of H_2O and the precipitate was filtered and washed thoroughly with H_2O, then dried to obtain 12.8 g of 14ß-hydroxy-7-Tes-baccatine III-1,14 carbonate with the same characteristics as those reported in example 1.

Example 5: <u>Synthesis of 13-(N-Boc-ß-isobutylserinyl)-14ß-hydroxybaccatine III, 1,14 carbonate</u>

Starting from 14ß-hydroxy-7-Tes-baccatine III-1,14 carbonate obtained as described in the above example, the procedure was as follows.

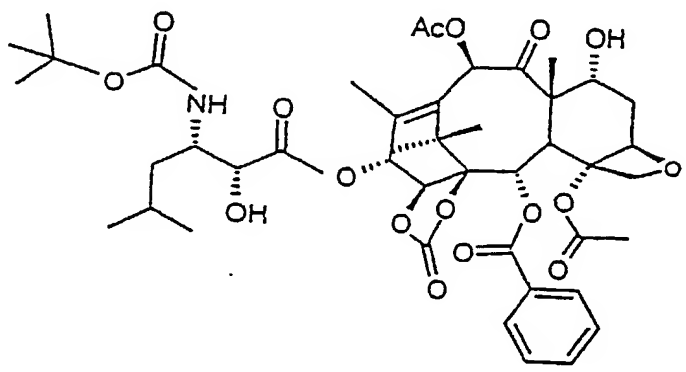
In a 1L round-bottom flask were placed 20 g of 14ß-hydroxy-7-Tes-1,14-carbonate-baccatine III together with 300 ml of strictly anhydrous toluene; 10 g of (4S, 5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid dissolved in CH_2Cl_2 and 2 g of N,N-dimethylaminopyridine (DMAP) were added and 9.5 g of dicyclohexylcarbodiimide (DCC) were added. The reaction

mixture was refluxed for 3h, then cooled to precipitate off the ureic product and mother liquors were washed with a $NaHCO_3$ saturated solution to remove the unreacted acid, then with diluted hydrochloric acid to remove DMAP and finally again with $NaHCO_3$ to neutrality. The organic phase was concentrated to dryness to obtain 41.5 g of product which could be directly used in the subsequent step.

40 g of this compound were deprotected in two steps by cleaving first Tes and then 2,4-dimethoxybenzaldehyde. 40 g of the compound were dissolved in 100 ml of an acetonitrile/pyridine mixture (80:100) under nitrogen and the mixture was cooled to 0°C; 13 ml of pyridinium fluoride were added and the whole was left under stirring for 24 h. The solution was poured into 2L of water and the product was filtered and dried under vacuum. The residue was dissolved in 60 ml of methylene chloride and this solution was added with 40 ml of Methanol HCl 0.6N under strong stirring and at 0°C. The reaction mixture was left for 2h under stirring, then diluted with 150 ml of methylene chloride and shaken with a $NaHCO_3$ solution adjusting pH to 6-7. The organic phase was concentrated to dryness and the residue was crystallized from acetone hexane, then dried to obtain 16.5 g of 13-(N-Boc-ß-isobutylisoserinyl)-14ß-hydroxybaccatine III 1,14-carbonate.

CLAIMS

1. Compound of formula (I):



(I)

2. A process for the preparation of the compound of formula (I), in which 13-(N-Boc-ß-isobutylisoserinyl)-14ß-hydroxy-baccatine III 1,14-carbonate is either treated with DBU (diazabicyclo[5,4,0] 7-undecene) in methanol or THF, or alternatively is left in solution with methylene chloride or chlorinated solvents in the presence of aliphatic alcohols selected from methanol, ethanol or propanol and with basic allumine, for a time ranging from one hour to 14 days.

3. A process for the preparation of 13-(N-Boc-ß-isobutylisoserinyl)-14ß-hydroxy-baccatine III or V 1,14-carbonate, which comprises the following steps:

a) transformation of 14ß-hydroxy-10-deacetylbaccatine III or V into the triethylsilylated deriyative at the 7-position;

b) preparation of the 1,14 carbonate derivative from the product of step (a);

c) selective acetylation of the hydroxyl at 10;

d) reaction of the product of step (c) with (4S,5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-ca

e) cleavage of the triethylsilyl and dimethoxybenzylidene protective groups from the product of step (d).

4. A process as claimed in claim 3, in which: the silylating agent of step (a) is triethyl chlorosilane; the 1,14 carbonate derivative in step (b) is prepared using phosgene in toluene in methylene chloride/pyridine 3:1 solution under nitrogen atmosphere; the reduction of step (c) is carried out with LiHMDS in anhydrous THF, and the resulting 10-hydroxy derivative is subsequently acetylated with acetyl chloride; the condensation reaction of step (d) is carried out in anhydrous apolar organic solvent, in the presence of a base and of the condensing agent dicyclohexylcarbodiimide (DCC); the triethylsilyl protective group in step (e) is removed with pyridinium fluoride in acetonitrile/pyridine solution under nitrogen, and the dimethoxybenzylidene protective group is removed in methylene chloride solvent by addition of HCl in methanol and subsequently of $NaHCO_3$.

5. A process for the preparation of 13-(N-Boc-ß-isobutylisoserinyl)-14ß-hydroxy-baccatine III or V 1,14-carbonate, which comprises the following steps:

a') selective acetylation of the hydroxyl at C-10 of 14ß-hydroxy-10-deacetylbaccatine III or V;

b') preparation of the 1,14 carbonate derivative from the product of step (a');

c') silylation of the hydroxyl at C-7;

d') reaction of the product of step (c) with (4S,5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid;

e') cleavage of the triethylsilyl and dimethoxybenzylidene protective groups from the product of step (d').

6. A process as claimed in claim 5, in which the selective acetylation of step (a') is carried out with scandium,

ytterbium salts, preferably $CeCl_3 \cdot 7H_2O$, and steps (b')-(e') are carried out analogously to steps (b), (a), (d) and (e) of claim 4.

7. A process for the preparation of (4S, 5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid, which comprises the following steps:

a) protection of the amino group of leucinol with Boc;

b) transformation of N-Boc-L-leucinol into N-Boc-L-leucinal;

c) preparation of the cyanhydrin of the product of step (b);

d) transformation of the cyanhydrine nitrile into the corresponding carboxylic acid;

e) formation of the carboxylic acid methyl ester;

f) purification of the (2R, 3S)-3-(N-Boc)amino-2-hydroxy-5-methylhexanoic acid methyl ester;

g) condensation of the product of step (f) with 2,4-dimethoxybenzaldehyde dimethyl acetal;

h) transformation of (4S, 5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid methyl ester into the corresponding carboxylic acid.

8. The following synthesis intermediates: 14ß-hydroxy baccatine III or V, 14ß-hydroxy baccatine III or V 1,14 carbonate, 14-ß-hydroxy-7-Tes-10-deacetylbaccatine III or V, 14-ß-hydroxy-7-Tes-baccatine ~~III or~~ V, 14-ß-hydroxy-7-Tes-baccatine III or V 1,14-carbonate, (4S,5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-1-oxazolidine-5-carboxylic acid.

9. Pharmaceutical compositions containing compound (I) together with pharmaceutically acceptable carriers and excipients.

10. The use of compound (I) for the preparation of a drug with anticancer activity.

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(54) Title: TAXANE DERIVATIVES AND PROCESSES FOR THE PREPARATION THEREOF

(57) Abstract: A novel taxane derivative with anticancer activity, a process for its preparation and a process for the preparation of 14-β-hydroxy-1,14-carbonate-baccatine III and V derivatives 13-substituted by an isoserine residue.

PENNIE & EDMONDS LLP DOCKET NO. 7914-085

DECLARATION FOR NON-PROVISIONAL PATENT APPLICATION*

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below at 201 et seq. beneath my name.

I believe I am the original, first and sole inventor if only one name is listed at 201 below, or an original, first and joint inventor if plural names are listed at 201 et seq. below, of the subject matter which is claimed and for which a patent is sought on the invention entitled

TAXANE DERIVATIVES AND PROCESSES FOR THE PREPARATION THEREOF

and for which a patent application:

☒ was filed in the United States on as Application No. 10/019,252
☒ was filed as PCT international Application No. PCT/EP00/06185 on July 3, 2000 and was amended under PCT Article 19 on *(if applicable)*

I hereby state that I have reviewed and understand the contents of the above identified application, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED PRIOR TO THE FILING DATE OF THE APPLICATION			
APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
MI99A001483	ITALY	06/July/99	YES ☒ NO ☐
			YES ☐ NO ☐
			YES ☐ NO ☐

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

PROVISIONAL APPLICATION NUMBER	FILING DATE

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information known to me which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

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		PATENTED	PENDING	ABANDONED

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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		SIGNATURE OF INVENTOR 205		DATE

POWER OF ATTORNEY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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AND EXCLUSION OF INVENTOR(S) UNDER 37 C.F.R. 3.71

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

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An assignment of the entire interest in the above-identified subject application:

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